Attorney's Docket No.: 13235-004002 / 00960-

562DIVFWC

Applicant: Huston et al.
Serial No.: 08/014,096
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Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1-46. (Cancelled)

47. (Currently Amended) A fused polypeptide produced by an organism by expression of a recombinant DNA, said polypeptide comprising a combination of:

a first sequence of amino acids comprising a leader sequence,
a hinge region, and at least one amino acid defining a cleavage site recognizable and cleavable
by a selected enzymatic cleavage agent, said cleavage site being outside of said hinge region,
said hinge region being a cysteine-free flexible amino acid sequence not normally associated
with said leader sequence and comprising at least two amino acids defining a secondary structure
which promote cleavage by said cleavage agent at said cleavage site, and
a second sequence of amino acids linked to said first sequence defining a selected target
polypeptide, whereby said cleavage site is a favored site for cleavage upon treatment of said
fused polypeptide with said cleavage agent when said fused polypeptide is disposed in solution
and said second amino acid sequence defining said selected target polypeptide is disposed in its
three-dimensional conformation.

- 48. (Original) The fused polypeptide of claim 47 wherein said leader sequence is adapted to facilitate concentration of said fused polypeptide.
- 49. (Original) The fused polypeptide of claim 48 wherein said leader sequence comprises an amphiphilic helix.

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50. (Original) The fused polypeptide of claim 47 wherein said hinge region comprises at least one proline residue.

- 51. (Original) The fused polypeptide of claim 47 wherein said hinge region comprises an amino acid sequence which forms a random coil when said fused polypeptide is disposed in aqueous solution.
- 52. (Original) The fused polypeptide of claim 47 wherein said hinge region includes a member selected form the group consisting of aspartic acid, glutamic acid, lysine, arginine, serine, threonine, proline, and combinations thereof in amounts sufficient to render said hinge region soluble in water.
- 53. (Original) The fused polypeptide of claim 47 wherein said hinge region comprises: a flexible cysteine-free amino acid sequence not normally associated with said leader sequence or said selected target polypeptide.
- 54. (Cancelled)
- 55. (Cancelled)
- 56. (Original) The fused polypeptide of claim 47 wherein said cleavage site is rendered preferentially accessible to said cleavage agent by said hinge region, thereby promoting preferential cleavage of said target polypeptide from said first sequence at said cleavage site in an environment in which said target polypeptide is disposed in its three dimensional conformation.
- 57. (Original) The fused polypeptide of claim 47 wherein said cleavage site is immediately adjacent said second amino acid sequence.

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58. (Original) The fused polypeptide of claim 47 wherein said cleavage site comprises one or a sequence of amino acids absent from the sequence comprising said target polypeptide.

- 59. (Original) The fused polypeptide of claim 47 wherein said cleavage side comprises a unique one or sequence of amino acids in said fused polypeptide.
- 60. (Original) The fused polypeptide of claim 47 wherein said cleavage site comprises a Glu residue.
- 61. (Original) The fused polypeptide of claim 60 wherein said cleavage site is cleaved by S. aureus V-8 protease.
- 62. (Cancelled)
- 63. (Original) The fused polypeptide of claim 47 wherein said target polypeptide is selected from the group consisting of growth factors, hormones, lymphokines, enzymes, antibody binding sites, viral proteins, non-enzymatically active prokaryotic proteins, and analogs thereof.
- 64. (Currently Amended) A fused polypeptide produced by an organism by expression of a recombinant DNA, said fused polypeptide encoded by a recombinant DNA comprising a combination of:

a first DNA segment encoding a sequence of amino acids comprising a leader sequence, a hinge region, and at least one amino acid defining a cleavage site recognizable and cleavable by a selected enzymatic agent, said cleavage site being outside said hinge region, said hinge region being a cysteine-free flexible amino acid sequence not normally associated with said leader sequence and comprising at least two amino acids defining a secondary structure which can promote cleavage by said cleavage agent at said cleavage site; and

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a second DNA segment linked to said first segment encoding a sequence of amino acids defining a selected target polypeptide, whereby said cleavage site is a favored site for cleavage upon treatment of said fused polypeptide with said cleavage agent when said fused polypeptide is disposed in solution and said amino acid sequence defining a target polypeptide is disposed in its three dimensional conformation.

- 65. (Original) The fused polypeptide of claim 64 wherein the recombinant DNA encoding said first DNA segment comprises DNA encoding a leader sequence comprising an amino acid sequence which imparts a preselected property to said fused polypeptide operable to facilitate concentration of said fused polypeptide.
- 66. (Original) The fused polypeptide of claim 64 wherein the recombinant DNA encoding said cleavage site comprises DNA encoding one or a sequence of amino acids absent from said sequence defining said target polypeptide.
- 67. (Original) The fused polypeptide of claim 64 wherein the recombinant DNA encoding said hinge region comprises DNA encoding a flexible cysteine-free amino acid sequence not normally associated with said leader sequence or said selected target polypeptide.
- 68. (Original) The fused polypeptide of claim 64 wherein the recombinant DNA encoding said second segment comprises a sequence of amino acids defining growth factors, hormones, lymphokines, enzymes, antibody binding sites, viral proteins, non-enzymatically active procaryotic proteins, and analogs thereof.
- 69. (Previously Presented) The fused polypeptide of claim 64 in which at least one of the two amino acids is proline.